

# Successful pseudorabies vaccination in maternally immune piglets using recombinant vaccinia virus vaccines

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## SUMMARY

Three gilts were vaccinated with a NYVAC vaccinia recombinant expressing glycoprotein gD of pseudorabies virus (PRV) (NYVAC/gD). After farrowing, the piglets were allowed to nurse normally to obtain colostral immunity and then were divided into four groups, receiving NYVAC/gD, a NYVAC recombinant expressing glycoprotein gB of PRV (NYVAC/gB), an inactivated PRV vaccine (iPRV), or no vaccine. The piglets were vaccinated twice, three weeks apart beginning at approximately two weeks of age and later challenged with virulent PRV oronasally. Piglets that received NYVAC/gB or iPRV were the best protected based on lack of mortality, lower temperature responses, decreased weight loss and decreased viral shedding after challenge. These results indicate effective strategies for stimulating active immune response while still under the protection of maternal immunity.

PSEUDORABIES (PR) is an enzootic disease in many parts of the world which causes mortality of newborn pigs, respiratory disease and in some cases maternal reproductive failure (Kluge et al 1992). The disease is thought to be perpetuated through latent viral infections from which the virus can occasionally reactivate and be shed back into the environment (Kluge et al 1992).

Vaccines have traditionally been important in controlling the signs of infection with PR virus (PRV). Although vaccines do not prevent replication or latent infection after experimental nasal challenge, studies have shown that vaccination decreases viral shedding and increases the exposure necessary to initiate an infection (Kluge et al 1992). Thus, vaccines can play an important role in eradication programs by helping decrease the transmission of the virus. The role of vaccines was further bolstered with the advent of gene deletion or marker vaccines that can differentiate vaccinated from naturally infected animals.

There is still one aspect of vaccination programs for pseudorabies that is a problem. Sows can be protected through vaccination and pass maternal antibodies to their piglets in their colostrum protecting them for a period of time. Unfortunately, maternally derived immunity also interferes with active immunisation of the piglets until it decreases to a level that often leaves the piglets unprotected. In past experiments, no vaccination schemes have been totally able to overcome the suppression of an active immune response caused by passively acquired immunity (De Leeuw et al 1982, De Leeuw and Van Oirschot 1985, De Smet et al 1994, Kit et al 1993, McCaw and Xu 1993, Van Oirschot and De Leeuw 1985, Van Oirschot 1987, 1991, Vannier 1985, 1986).

The purpose of this study was to determine if piglets could be actively immunised with recombinant vaccinia virus PR vaccines or an inactivated PRV vaccine after nursing immune sows which had received a recombinant vaccinia virus vaccine. Three different vaccines were chosen to give to the piglets. The NYVAC vaccinia virus recombinant expressing glycoprotein gD of PRV (NYVAC/gD) was given to the dams as well as piglets because in past studies it

induced high virus neutralising (VN) antibody titres and protected well when given to seronegative pigs (Brockmeier et al 1993). With the possibility that the immune response to gD would be blocked by the passively acquired antibodies from the dam, a NYVAC recombinant expressing glycoprotein gB of PRV (NYVAC/gB) was also chosen. This would also give insight as to whether passively acquired antibodies to the vaccinia vector would interfere with the active immune response in the piglets. And finally an inactivated PRV vaccine (iPRV) was chosen to see if a protective immune response could be induced in the piglets with a full complement of PRV proteins when antibody to just gD was present.

## MATERIALS AND METHODS

### Experimental design

Three gilts were vaccinated with NYVAC/gD six and two weeks prior to their predicted farrowing dates and two gilts were left as non-vaccinated controls. After farrowing, the piglets were allowed to nurse normally to obtain colostral immunity and weaned at four weeks of age. The piglets from each litter were randomly divided into four groups, and received either NYVAC/gD, NYVAC/gB, iPRV, or no vaccine. Group identification, number of piglets per group and vaccine treatment is summarised in Table 1. Two injections of each vaccine were given to the piglets, the first at approxi-

TABLE 1: Experimental groups (number of animals)

| Gilt group | Vaccine      | Piglet group | Vaccine      |
|------------|--------------|--------------|--------------|
| 1          | NYVAC/gD (3) | 1a           | NYVAC/gD (6) |
|            |              | 1b           | NYVAC/gB (6) |
|            |              | 1c           | iPRV (6)     |
|            |              | 1d           | None (6)     |
| 2          | None (2)     | 2a           | NYVAC/gD (5) |
|            |              | 2b           | NYVAC/gB (5) |
|            |              | 2c           | iPRV (5)     |
|            |              | 2d           | None (4)     |

mately two weeks of age and the second three weeks after the first. The gilts were housed in isolation facilities and then removed at the time of weaning. The piglets were left in the isolation rooms in which they farrowed until the end of the experiment. Based on past studies we have found no transmission of the NYVAC vector to occur between vaccinated and non-vaccinated animals (Mengeling et al 1994a and unpublished data).

The piglets were challenged with virulent PRV oronasally, five weeks after the second vaccination. After challenge the piglets were monitored daily for the next 11 days for clinical signs and rectal temperatures. The piglets were weighed 10 days prior to challenge, on the day of challenge, and on days 3, 7, 10, and 17 after challenge. Oropharyngeal swabs were taken just prior to challenge and on days 1, 3, 5, 7, 9 and 11 after challenge to determine the extent of viral shedding.

Serum samples were obtained from the gilts prior to vaccination, four weeks after the first vaccination and 24 hours after farrowing. Serum samples from the piglets were obtained prior to vaccination, three weeks after the first vaccination, five weeks after the second vaccination (just prior to challenge) and 17 days after challenge.

Differences between experimental groups with regard to VN antibody titres and weight responses on day 7 after challenge with virulent virus were analysed statistically, using Student's *t* test.

#### Virus and cells

The inactivated PRV vaccine used to vaccinate the piglets was the commercial product PRV/Marker Gold-KV (SyntroVet Inc.) which has deletions in its gE and gG glycoprotein genes. A 2 ml dose was administered intramuscularly.

The NYVAC strain of vaccinia virus is an attenuated vaccinia virus vector derived by genetically engineering the deletion of putative virulence and host range genes from the Copenhagen vaccinia virus vaccine strain (Tartaglia et al 1992). A 1 ml dose of  $10^{7.5}$  median cell culture infective doses (CCID<sub>50</sub>) was administered intramuscularly to the gilts (NYVAC/gD) or piglets (NYVAC/gB or NYVAC/gD). NYVAC-based viruses were propagated in primary chicken embryo fibroblasts and titrated on bovine embryonic spleen cells (BESp) using CCID<sub>50</sub> as described previously (Brockmeier et al 1993).

Two ml of the Indiana-Funkhauser strain of PRV, containing  $10^8$  plaque forming units ml<sup>-1</sup>, were administered oronasally to challenge immunity of the piglets after vaccination. An established porcine kidney cell line, PK-15, was used to propagate and titrate PRV challenge virus and PRV titrations were performed by plaque assay (Mengeling 1991).

#### Virus isolation and antibody titration

Virus isolation and titration of PRV from oropharyngeal swabs were performed as described previously using BESp and PK-15 cells respectively (Brockmeier et al 1993).

Titration of VN antibodies in the serum of pigs was performed using PK-15 cells as previously described (Mengeling 1991).

Sera from all piglets collected prior to vaccination, five weeks after the second vaccination, and 17 days after challenge were tested for antibodies to PRV glycoprotein gE using the Herdcheck: Anti-PRV-gI(gE) test kit (IDEXX Laboratories Inc).

## RESULTS

### Antibody response

All gilts were free of VN antibody to PRV prior to vaccination and the non-vaccinated gilts remained PRV antibody negative throughout the experiment. The three vaccinated gilts had VN titres of 256, 1024 and 4096 after farrowing. The VN antibody titres at the time of vaccination in piglets born to vaccinated dams ranged from 128 to 8192 and the geometric mean titres for the groups of these piglets ranged from  $2^{9.5}$  to  $2^{10}$  (Table 2). All piglets born to non-vaccinated dams had no detectable VN antibody to PRV at the time of vaccination. All piglets remained clinically normal following both injections.

Three weeks after the first vaccination, all VN antibody titres in the piglets with maternal immunity were lower than on the day of vaccination and there were no significant differences between vaccinated and non-vaccinated groups of piglets (Table 2). Of the seronegative piglets, group 2c had VN antibody titres which ranged from 32 to 256 after the first vaccination, three of the five piglets in group 2a responded after the first vaccination with titres of 2, and none of the piglets of group 2b had titres after the first vaccination. Group 2d piglets remained seronegative until challenge.

With regard to the piglets with maternal immunity, antibody titres after the second vaccination were, on average, lower for all groups compared to the titres prior to the second vaccination. However, the vaccinated piglets in group 1a and 1c had significantly higher mean antibody responses than did their non-vaccinated counterparts in group 1d ( $P<0.05$ ) (Table 2). The mean titre of piglets in group 1b was higher than that of group 1d but not significantly higher ( $P<0.1$ ). The titres of group 2a and 2c, were significantly higher than the titres of group 1a and 1c ( $P<0.01$ ). Group 1b piglets had significantly higher mean antibody response than group 2b piglets ( $P<0.01$ ).

The antibody titres for all piglets were higher after challenge. The titres for the vaccinated pigs were greater than the non-vaccinated pigs and were highest for the piglets vaccinated with NYVAC/gD (Table 2).

### Challenge

All pigs developed clinical signs of pseudorabies which ranged from mild upper respiratory signs to severe respiratory distress, anorexia, lethargy, vomiting and seizures. The

TABLE 2: Log<sub>2</sub> geometric mean PRV neutralising antibody titres of piglets born to vaccinated and non-vaccinated gilts (range)

| Piglet vaccine | Maternal immunity | Day 0 <sup>a</sup> | Day 21 <sup>b</sup> | Day 56 <sup>c</sup> | Day 73 <sup>d</sup> |
|----------------|-------------------|--------------------|---------------------|---------------------|---------------------|
| NYVAC/gD       | Yes               | 10.0(8-13)         | 8.3(7-10)           | 6.2(4-8)            | 16.5(15-19)         |
|                | No                | <1                 | 0.5(<1-1)           | 10.2(10-11)         | 18.0(18)            |
| NYVAC/gB       | Yes               | 10.0(8-13)         | 8.0(7-10)           | 5.3(3-6)            | 15.5(12-18)         |
|                | No                | <1                 | <1                  | 3.2(2-4)            | 14.6(12-17)         |
| iPRV           | Yes               | 9.5(7-11)          | 7.8(6-10)           | 6.3(6-7)            | 14.0(13-15)         |
|                | No                | <1                 | 6.6(5-8)            | 11.2(10-12)         | 16.0(15-18)         |
| None           | Yes               | 9.8(9-13)          | 7.5(6-10)           | 3.8(2-6)            | 11.0(11)            |
|                | No                | <1                 | <1                  | <1                  | 10.7(10-12)         |

<sup>a</sup> Pre-vaccination

<sup>b</sup> Twenty-one days after first vaccination

<sup>c</sup> Thirty-five days after second vaccination

<sup>d</sup> Seventeen days after challenge

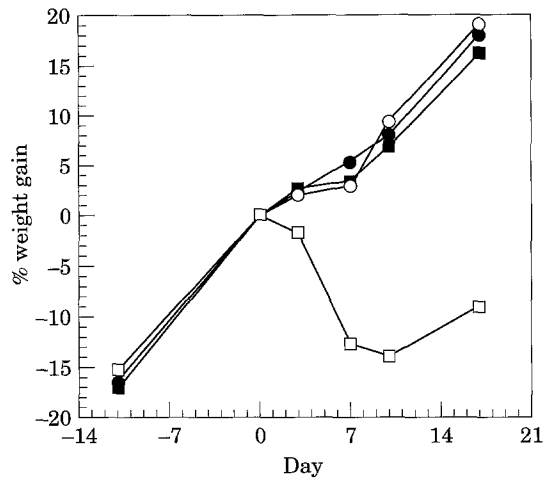


FIG 1: Mean percent weight gain before and after challenge with virulent PRV in seronegative piglets vaccinated with NYVAC/gD, NYVAC/gB, iPRV or not vaccinated. Key: —●— piglet 2a; —○— piglet 2b; —■— piglet 2c; —□— piglet 2d

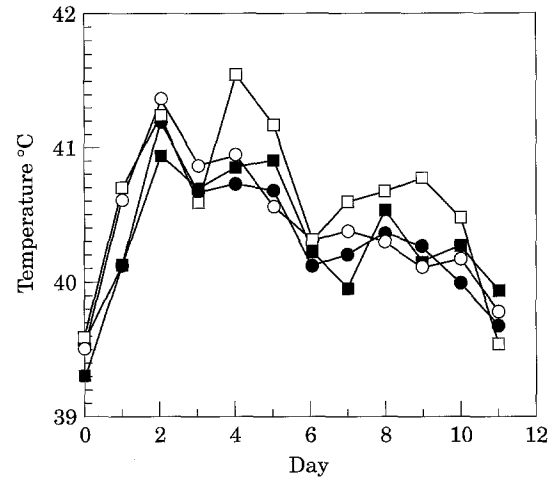


FIG 2: Mean temperature response after challenge with virulent PRV in seronegative piglets vaccinated with NYVAC/gD, NYVAC/gB, iPRV or not vaccinated. Key: —●— piglet 2a; —○— piglet 2b; —■— piglet 2c; —□— piglet 2d

severity of clinical signs varied among the groups, with group 1a and the non-vaccinated groups 1d and 2d experiencing the most severe signs while the other vaccinated groups tended to have milder signs of disease. Group 1a had two piglets die, one on day 6 and the other on day 14 after challenge. Group 1d had five piglets die; one on day 5, two on day 6 and two on day 11 after challenge. Group 2d had one pig die on day 8 after challenge.

All vaccines were similarly protective when given to piglets that were seronegative prior to vaccination. Group 2a, 2b and 2c piglets, on average, did not lose weight after challenge and weight gains were significantly better compared with group 2d piglets which lost considerable weight and had still not, as a group, regained their pre-challenge weight by the end of the experiment (Fig 1). Group 2a, 2b and 2c piglets had lower temperature responses and shed less virus after day 3 post challenge than did their non-vaccinated counterparts in group 2d (Figs 2 and 3).

After challenge, piglets in group 1a which had maternal immunity and were vaccinated with NYVAC/gD did not perform as well as piglets in 2a which were seronegative piglets vaccinated with the same vaccine. In fact, there was

no significant difference in the average weight loss between groups 1a and 1d and temperature response of piglets in group 1a was similar to that of non-vaccinated piglets (Figs 4 and 5). The piglets of group 1a did shed less virus after day 5 post challenge than did non-vaccinated piglets (Fig 6). Piglets vaccinated with NYVAC/gB or iPRV which had prior maternal immunity (groups 1b and 1c) had a lower temperature response, significantly less weight loss ( $P < 0.02$ ), and shed less virus after day 3 post challenge, on average, than non-vaccinated piglets (Figs 4, 5 and 6). The temperature response, weight response, and viral shedding pattern of the piglets in groups 1b and 1c were similar to those of groups 2b and 2c which were seronegative piglets which received these vaccines (Figs 1-6) and there was no difference between 1b and 1c in providing protection. There also was no difference in severity of disease between the two non-vaccinated groups 1d and 2d.

#### Differential gE antibody testing

All piglets tested negative for antibody to gE prior to vaccination and after the second vaccination. All piglets tested positive for antibody to gE after challenge.

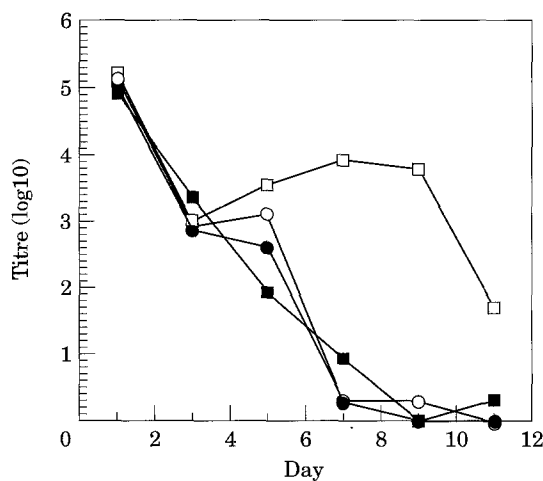


FIG 3: Mean viral shedding curves after challenge with virulent PRV in seronegative piglets vaccinated with NYVAC/gD, NYVAC/gB, iPRV or not vaccinated. Key: —●— piglet 2a; —○— piglet 2b; —■— piglet 2c; —□— piglet 2d

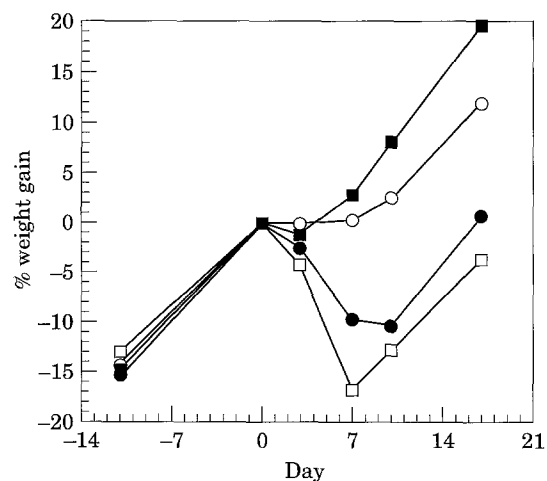


FIG 4: Mean percent weight gain before and after challenge with virulent PRV in maternally immune piglets vaccinated with NYVAC/gD, NYVAC/gB, iPRV or not vaccinated. Key: —●— piglet 1a; —○— piglet 1b; —■— piglet 1c; —□— piglet 1d

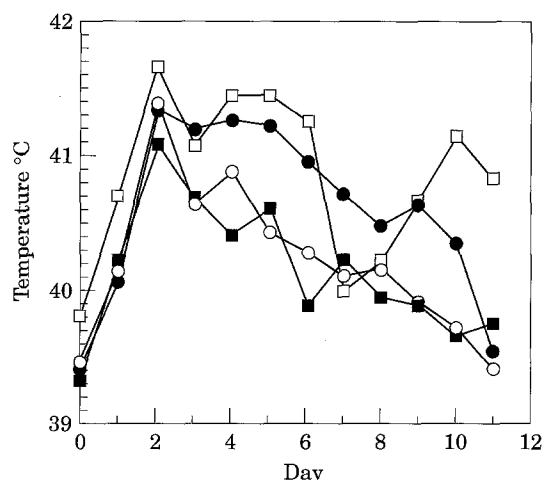


FIG 5: Mean temperature response after challenge with virulent PRV in maternally immune piglets vaccinated with NYVAC/gD, NYVAC/gB, iPRV or not vaccinated. Key: ●—piglet 1a; ○—piglet 1b; ■—piglet 1c; □—piglet 1d

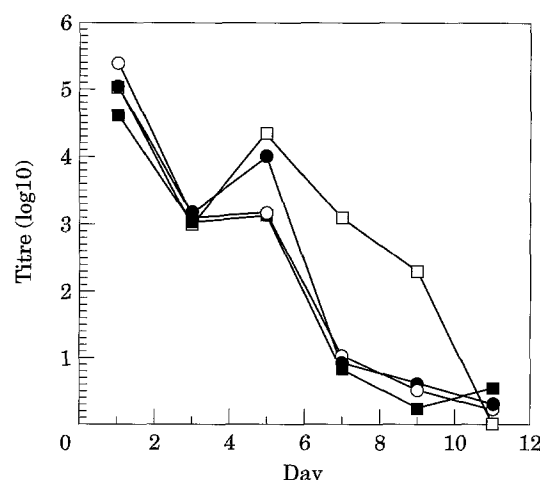


FIG 6: Mean viral shedding curves after challenge with virulent PRV in maternally immune piglets vaccinated with NYVAC/gD, NYVAC/gB, iPRV or not vaccinated. Key: ●—piglet 1a; ○—piglet 1b; ■—piglet 1c; □—piglet 1d

### DISCUSSION

The purpose of this experiment was to determine if recombinant vaccinia virus vaccines or a conventional vaccine could be used to vaccinate piglets with maternally derived immunity from dams vaccinated with one of the recombinant vaccines. In past experiments, no vaccination schemes have been totally able to overcome the suppression of an active immune response caused by passively acquired immunity (De Leeuw et al 1982, De Leeuw and Van Oirschot 1985, De Smet et al 1994, Kit et al 1993, McCaw and Xu 1993, Van Oirschot and De Leeuw 1985, Van Oirschot 1987, 1991, Vannier 1985, 1986). Intranasal vaccination of maternally immune piglets with attenuated strains of PRV has induced partial protection but it is less than the protection following administration of vaccine to seronegative pigs (De Leeuw et al 1982, De Leeuw and Van Oirschot 1985, McCaw and Xu 1993, Van Oirschot and De Leeuw 1985, Van Oirschot 1987, 1991, Vannier 1986). This makes it difficult to know when to vaccinate piglets to get an adequate active immune response. The higher the level of passively acquired antibodies, the greater the suppression and by the time the maternal antibody levels have decreased to a point where conventional vaccines are effective at inducing active immune responses, the piglets are probably no longer passively protected. This leaves a window of time when the animals are susceptible to disease.

In this experiment several vaccination strategies were attempted to try to actively vaccinate piglets while still under the protection of high maternally derived antibody titres. We vaccinated pregnant gilts with a NYVAC vaccinia vector expressing gD, and then vaccinated the piglets with either inactivated PRV vaccine or NYVAC vectors containing either gB or gD of PRV. As expected from past experiments using the NYVAC/gD vaccine, this vaccine induced high VN antibody responses in the gilts which were passed to the piglets. We vaccinated the piglets twice because we know from previous experiments that in the case of the NYVAC vectors there must be a priming of the immune system from the first injection to get a measurable response with the second injection (Brockmeier et al 1993).

This is not the case with the iPRV vaccine, where one vaccination is capable of stimulating a measurable immune response.

The piglets with maternal immunity which were vaccinated with NYVAC/gD had significantly higher VN antibody titres than maternally immune non-vaccinated piglets but the titres were significantly lower than in seronegative animals vaccinated with this vaccine. This suggests that there was an active immune response but it was suppressed by the presence of maternal immunity. Upon challenge, NYVAC/gD given to seronegative piglets protected well, whereas it failed to protect adequately in maternally immune piglets. It is probable that the immune response to gD was diminished by antibodies to gD passed to piglets in the colostrum of the gilts. We have found gD to be an especially good inducer of virus neutralising antibodies and we have not found a diminished response to NYVAC recombinants even when animals with existing NYVAC antibody titres were vaccinated multiple times (Brockmeier et al 1993, Mengeling et al 1994b).

Piglets with maternal immunity vaccinated with iPRV had a significantly higher mean antibody response than non-vaccinated maternally immune piglets but also had a significantly lower VN antibody response as compared with seronegative piglets given this vaccine. Unlike the NYVAC/gD vaccinated piglets, these piglets were protected much better against virulent challenge. The antibody response to gD may have been inhibited in this case, explaining the decreased VN antibody titres, but the other antigens of the virus are sufficient enough to induce a protective response. Another possibility is that the initial vaccination with this vaccine was blocked and that the second vaccination given when the piglets were approximately five weeks of age was more effective at stimulating an active immune response. The antibody titres induced in seronegative pigs after one injection of iPRV are similar to those seen after the second injection in the pigs in this experiment. However, the antibody titres at the time of the second injection were still at a level that under most circumstances interferes with active immunity.

Maternally immune piglets vaccinated with NYVAC/gB had VN antibody titres slightly higher than their non-vaccinated counterparts and significantly higher than the seronegative piglets given this vaccine. The higher titres in maternally immune vaccinated piglets may be due to the combination of anti-gD antibodies that were maternally derived and the anti-gB antibodies produced in response to

the vaccine. Both seronegative and maternally immune piglets vaccinated with NYVAC/gB were similarly well protected from virulent challenge with PRV. There appeared to be no inhibition of the immune response due to any passively acquired antibodies to the vaccinia vector and since two different antigens of PRV were used, there should be active immunisation with the second antigen.

VN antibody titres at the time of challenge did not correlate well with the degree of protection. For example, all three vaccines protected similarly when given to seronegative pigs, yet the geometric mean titres in these groups ranged from 3.2 to 11.2. Also, even though the maternally immune piglets which received NYVAC/gD had similar titres to the maternally immune piglets which received iPRV, and slightly higher titres than the piglets receiving NYVAC/gB, they were not as well protected as the latter groups. Curiously, all the non-vaccinated piglets born to immune dams had VN titres at the time of challenge which were comparable with the seronegative group receiving NYVAC/gB and yet this group of piglets was the most severely affected in terms of mortality, with five of the six pigs succumbing to the disease. These differences may indicate different immune mechanisms induced by the different antigens of PRV. The PRV glycoprotein gD appears to be a good inducer of neutralising antibodies and it may take high levels of these antibodies to be protective. It is even possible that low levels of these antibodies were detrimental, such as is seen in cases of antibody dependent enhancement of disease. Glycoprotein gB on the other hand seems to induce protection even though only low levels of neutralising antibodies are present possibly indicating other immune mechanisms such as antibody dependent cytotoxicity or cell mediated immunity are induced by this protein.

The results of this study indicate that different protective antigens can be delivered to dams and their offspring through vaccinia vectored vaccines to circumvent the problems associated with active vaccination in the presence of maternally derived immunity. The compatibility of these vaccines with differential testing indicates the potential to use a combination of these two vaccinia recombinant PRV vaccines, or a recombinant and a conventional vaccine, to protect gilts and piglets alike from the effects of PRV infection, and reduce shedding of the virus. The principles of delivering different combinations of protective antigens to dam and offspring could be applied to other diseases as well.

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